

WHAT IS CLAIMED IS:

- 1 1. A method of detecting a target nucleic acid in a sample, comprising:
2 a) contacting the sample with at least one AP site probe and an AP endonuclease, under
3 conditions sufficient to allow the AP site probe to hybridize to the target nucleic acid
4 and form a reaction mixture, wherein said AP site probe comprises an oligonucleotide
5 NA that hybridizes to the target nucleic acid and a functional tail R comprising a
6 detectable reporter group, said functional tail R attached via a phosphodiester bond of
7 a phosphate group to the 3' terminal nucleotide of the NA, wherein the reporter group
8 is not detected when the functional tail R is attached to the NA; and
9 b) incubating the reaction mixture under reaction conditions sufficient to allow said AP
10 endonuclease to cleave the phosphodiester bond attaching the functional tail R to the
11 3' terminal nucleotide of the NA, wherein the AP endonuclease preferentially cleaves
12 the phosphodiester bond attaching the tail R to the NA when the NA is hybridized
13 with a complementary target nucleic acid sequence in comparison to when the NA is
14 unhybridized or hybridized to a non-complementary nucleic acid; and
15 c) detecting the reporter group on the cleaved functional tail R, whereby the target nucleic
16 acid is detected.
- 1 2. The method of Claim 1, further comprising contacting the sample with
2 an enhancer oligonucleotide, wherein the 5'-end of said enhancer oligonucleotide hybridizes
3 to the target nucleic acid on the 3' side of the hybridized AP site probe, wherein a gap of 0-
4 5 unpaired bases resides between the enhancer oligonucleotide and the AP site probe
5 hybridization locations with the target nucleic acid.
- 1 3. The method of Claim 2, wherein the 5'-end of said AP site probe is
2 covalently linked to the 3'-end of said enhancer.
- 1 4. The method of Claim 1, further comprising a quencher molecule
2 attached to the 5' end of the NA of said AP site probe via a non-cleavable linker.
- 1 5. The method of Claim 1, wherein the cleavage of the phosphodiester
2 bond results in a hybridized NA having a free 3'-OH.
- 1 6. The method of Claim 5, further comprising contacting the sample with
2 a nucleic acid polymerase, and further comprising amplifying the target nucleic acid, said

3 amplifying comprising incubating the sample under reaction conditions sufficient to allow the
4 polymerase to extend the hybridized NA in a template-specific manner.

1 7. The method of Claim 6, wherein said amplifying is isothermal
2 amplification.

1 8. The method of Claim 5, wherein the sample is incubated under
2 reaction conditions that simultaneously allow the AP endonuclease to cleave the
3 phosphodiester bond of the AP site probe and the polymerase to extend the cleaved AP site
4 probe in a template-specific manner.

1 9. The method of Claim 1, wherein the NA of said AP site probe is 3-200
2 nucleotides in length.

1 10. The method of Claim 1, wherein the functional tail R is attached to the
2 phosphate group through a hydroxyprolinol linker.

1 11. The method of Claim 1, wherein the reporter group is a fluorophore.

1 12. The method of Claim 1, wherein the AP endonuclease is a Class II AP
2 endonuclease.

1 13. The method of Claim 12, wherein the Class II AP endonuclease is an
2 *E.coli* Endonuclease IV.

1 14. The method of Claim 1, wherein the target nucleic acid is attached to a
2 solid support.

1 15. The method of Claim 1, wherein the AP site probe is attached to a
2 solid support.

1 16. The method of Claim 2, wherein the enhancer is attached to a solid
2 support.

1 17. The method of Claim 1, wherein said at least one AP site probe
2 comprises a first AP site probe and a second AP site probe, wherein said first probe
3 comprises a NA portion comprising at least one base difference from the NA portion of said

4 second probe, and wherein said first probe comprises a reporter group that is distinguishably
5 detectable from the reporter group of said second probe.

1 18. The method of Claim 17, wherein the reporter group of said first probe
2 and said second probe comprises a fluorophore, and wherein the fluorophore of said first
3 probe comprises a distinguishably detectable emission wavelength from the fluorophore of
4 said second probe.

1 19. The method of Claim 17, wherein said at least one base difference
2 between the NA of said first probe and the NA of said second probe comprises a base
3 difference at position 1, 2, 3 or 4 from the 3' end of said probes.

1 20. The method of Claim 17, wherein said at least one base difference
2 between the NA of said first probe and the NA of said second probe comprises a base
3 difference at position 1 or 2 from the 3' end of said probes.

1 21. The method of Claim 1, wherein said at least one AP site probe
2 comprises a plurality of AP site probes, wherein the NA portion of said probes are members
3 of a universal library.

1 22. The method of Claim 21, wherein the NA portion of said AP site probe
2 members is 5-8 nucleotides in length.

1 23. The method of Claim 9, wherein said AP site probe members further
2 comprise at least one modified base.

1 24. A kit comprising an AP site probe for carrying out the method of
2 Claim 1.

1 25. A method of detecting a target nucleic acid in a sample, comprising:
2 a) contacting the sample with at least one AP site probe and an AP endonuclease, under
3 conditions sufficient to allow the AP site probe to hybridize to the target nucleic acid
4 and form a reaction mixture, wherein said AP site probe comprises an oligonucleotide
5 NA that hybridizes to the target nucleic acid, a functional tail R comprising a
6 quencher molecule, said functional tail R attached via a phosphodiester bond of a
7 phosphate group to the 3' terminal nucleotide of the NA, and a reporter group attached

8 via a non-cleavable linker to the 5' terminal nucleotide of the NA, wherein the
9 reporter group is not detected when the functional tail **R** is attached to the NA; and
10 b) incubating the reaction mixture under reaction conditions sufficient to allow said AP
11 endonuclease to cleave the phosphodiester bond attaching the functional tail **R** to the
12 3' terminal nucleotide of the NA, wherein the AP endonuclease preferentially cleaves
13 the phosphodiester bond attaching the tail **R** to the NA when the NA is hybridized
14 with a complementary target nucleic acid sequence in comparison to when the NA is
15 unhybridized or hybridized to a non-complementary nucleic acid; and
16 c) detecting the reporter group upon cleavage of the functional tail **R**, whereby
17 the target nucleic acid is detected.

1 26. A method of amplifying a target nucleic acid sequence in a sample
2 comprising:
3 a) contacting the sample with at least one forward primer and at least one reverse primer,
4 an AP endonuclease, and a nucleic acid polymerase, under conditions sufficient to
5 allow the forward and reverse primers to hybridize to the target nucleic acid and form
6 a reaction mixture, wherein the forward and the reverse primer independently
7 comprise a sequence structure $(NA_1-L)_m-NA_2$, wherein NA_1 and NA_2 are nucleic acid
8 sequences complementary to the target nucleic acid, **L** is an AP endonuclease-
9 cleavable linker, and **m** is from 0 to 100, wherein at least one of said forward and
10 reverse primer comprises an AP endonuclease-cleavable linker, **L**;
11 b) incubating the reaction mixture under reaction conditions that simultaneously allow the
12 AP endonuclease to cleave at a linker site **L**, thereby generating a free 3'-OH, and the
13 polymerase to extend the primers in a template-specific manner; whereby the target
14 nucleic acid sequence is amplified.

1 27. The method of Claim 26, wherein said amplifying is isothermal
2 amplification.

1 28. The method of Claim 26, wherein said AP endonuclease is an
2 Endonuclease IV.

1 29. The method of Claim 1, wherein said target nucleic acid is a product of
2 an amplification reaction.

1 **30.** The method of Claim **29**, wherein said amplification reaction is
2 polymerase chain reaction.

1 **31.** The method of Claim **29**, wherein said amplification reaction is
2 polymerase chain reaction and said method uses a thermostable endonuclease.

1 **32.** The method of Claim **29**, wherein said amplification reaction is an
2 isothermal amplification reaction.

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